PRODUCT INFORMATION

Thermo Scientific
MagJET Whole Blood Genomic DNA Kit
#K2741, #K2742

Read Storage information (p. 4) upon receipt and store the kit components appropriately!

www.thermoscientific.com/onebio

#__
Lot 00000000
Expiry Date 00.0000

CERTIFICATE OF ANALYSIS

Thermo Scientific™ MagJET™ Whole Blood Genomic DNA Kit is qualified by isolating genomic DNA from 200 µL of frozen human whole blood following the protocol outlined in the manual. The quality of isolated DNA is evaluated spectrophotometrically and by agarose gel electrophoresis. The purified DNA has an A_{260}/A_{280} ratio of 1.8±0.2. The functional quality of purified DNA is evaluated by digestion with restriction endonucleases.

Quality authorized by: Jurgita Žilinskienė

Rev.1 6666

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COMPONENTS OF THE KIT

MagJET Whole Blood Genomic DNA Kit	#K2741 96 preps	#K2742 384 preps
Proteinase K	2 × 1.0 mL	8 × 1.0 mL
Lysis Buffer for MagJET Blood gDNA Kit	15 mL	60 mL
MagJET Magnetic Beads	2 × 1.4 mL	10.6 mL
Binding Buffer (conc.) for MagJET Blood gDNA Kit	23 mL	90 mL
Wash Buffer 1 (conc.) for MagJET Blood gDNA Kit	100 mL	4 × 100 mL
Wash Buffer 2 (conc.) for MagJET Blood gDNA Kit	40 mL	2 × 40mL
Elution Buffer	30 mL	3 × 30mL

STORAGE

Proteinase K solution is stable at room temperature as long as the vial remains sealed. After opening, store at -20°C. MagJET Magnetic Beads should be stored at 4°C. Other components of the kit should be stored at room temperature (15-25°C).

DESCRIPTION

The MagJET Whole Blood Genomic DNA Kit is designed for fast and efficient purification of genomic DNA from fresh or frozen whole blood treated with EDTA or citrate, as well as blood fractions.

Note: Samples preserved with heparin are not compatible with this kit.

The kit utilizes paramagnetic bead technology enabling high yields and robust performance. High binding capacity, uniform particle size, and rapid magnetic response of MagJET magnetic beads makes the technology ideal for high throughput automatic nucleic acid purification, as well as for manual nucleic acid purification by low sample throughput users.

The resulting high quality DNA is free of proteins, nucleases and other contaminants or inhibitors, can be used in a wide range of downstream applications such as PCR, qPCR and other enzymatic reactions.

PRINCIPLE

The MagJET Whole Blood Genomic DNA Kit uses a highly efficient MagJET magnetic particle-based technology for nucleic acid purification. The whole nucleic acid isolation process combines simple steps of sample lysis, DNA binding to the magnetic beads, washing and elution.

Purification protocols optimized for automated KingFisher instruments utilize a high throughput magnetic bead transfer technique where magnetic beads are transferred through different reagent plates containing lysis, binding, wash and elution reagents. This enables high throughput nucleic acid purification and eliminates multiple pipetting steps.

Alternatively protocol is available where instead of magnetic particles, buffers and other reagents are transferred in each of the protocol steps, while magnetic beads remain captured on the wall of the tube with the help of a magnetic rack. This allows the kit to be used in various throughput applications using a magnetic rack and manual or automated pipetting equipment.

IMPORTANT NOTES

- Add the indicated volumes of ethanol (96-100%) to **Binding Buffer**, concentrated **Wash Buffer 1 and 2** prior to first use:

	96 preps			384 preps		
	Binding Buffer	**Wash Buffer 1**	**Wash Buffer 2**	**Binding Buffer**	**Wash Buffer 1**	**Wash Buffer 2**
Concentrated buffer	23 mL	100 mL	40 mL	90 mL	100 mL	40 mL
Ethanol (96-100%)	**23 mL**	**100 mL**	**200 mL**	**90 mL**	**100 mL**	**200 mL**
Total volume:	46 mL	200 mL	240 mL	180 mL	200 mL	240 mL

After preparing each solution, mark the bottle to indicate that this step has been completed.

- Check all solutions in the kit for any salt precipitation before each use. Re-dissolve any precipitates by warming the solution at 37°C, and then equilibrate to room temperature (15-25°C).
- Wear gloves when handling the **Lysis Buffer, Binding Buffer** and **Wash Buffer 1** as these reagents contain irritants (*see* page 22 for SAFETY INFORMATION).

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and pipette tips
- 1.5 mL tubes
- Disposable gloves
- 96-100% ethanol, molecular biology grade
- Automatic magnetic particle processor and consumables *or*
- Magnetic particle processing rack.

PROTOCOL SELECTION GUIDE

The MagJET Whole Blood Genomic DNA Kit provides optimized protocols for genomic DNA purification from different amounts of starting material (200 µL, 1 mL and 2 mL). The kit is compatible with automated and manual sample processing, allowing low- to high-throughput nucleic acid purification workflow.

The following selection guide summarizes available protocols depending on starting sample volume, throughput and sample processing type. Automation protocols are optimized for KingFisher Flex and KingFisher Duo instruments.

Protocol selection guide:

Sample type	Sample volume	Throughput per run	KingFisher Flex Instrument	KingFisher Duo Instrument	Manual processing	MagJET purification protocol	Page
Fresh or frozen whole blood treated with EDTA or citrate.	**200 µL**	96	●	-	-	**Protocol A**	page 7
		12	-	●	-	**Protocol D**	page 13
	1 mL	24	●	-	-	**Protocol B**	page 9
		6	-	●	-	**Protocol E**	page 15
	2 mL	24	●	-	-	**Protocol C**	page 11
		6	-	●	-	**Protocol F**	page 17
	200 µL	variable	-	-	●	**Protocol G**	page 19
Dried blood	**-**	variable	●	●	●	**Protocol H**	page 20
Buffy coat	**1.5 mL of whole blood**	variable	●	●	●	**Protocol I**	page 20
Bone marrow	**20 µL**	variable	●	●	●	**Protocol J**	page 20
Urine	**0.5 mL**	variable	●	●	●	**Protocol K**	page 21
Swabs	**-**	variable	●	●	●	**Protocol L**	page 21

GENOMIC DNA PURIFICATION PROTOCOLS AND PIPETTING INSTRUCTIONS

Protocols A-G (pages 7-19) are recommended for genomic DNA purification from fresh or frozen whole blood treated with EDTA or citrate. **Note:** Samples preserved with heparin are not compatible with the kit. For genomic DNA purification from dried blood, buffy coat, bone marrow, urine or swabs follow **Protocols H-L** (pages 20-21).

Protocol A. Instructions for genomic DNA purification from 200 µL of whole blood using KingFisher Flex 96 and Microtiter deep well 96 plates

Note:

- When using the MagJET Whole Blood Genomic DNA Purification Kit for the first time, prepare working solutions of Wash Buffer 1, Wash Buffer 2 and Binding Buffer as described on page 5.
- Transfer the **Blood_gDNA_200uL_Flex** protocol file to the KingFisher instrument before first use. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the BindIt Software for KingFisher Instruments version 3.1 User Manual. The protocol files for MagJET Whole Blood Genomic DNA Kit can be found on product web page on www.thermoscientific.com/onebio

1. Obtain four empty Thermo Scientific Microtiter deep well 96 plates and two empty Thermo Scientific KingFisher Flex 96 KF plates.
2. Prepare the **Sample** plate (Microtiter deep well 96 plate).

Add the following reagents to the Sample plate and leave the plate at room temperature while the other plates are being filled.

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	Microtiter deep well 96 plate	Sample	Blood sample	200 µL
			Lysis Buffer	100 µL
			Proteinase K	20 µL

3. Prepare the other plates as follows:

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
2	Microtiter deep well 96 plate	Wash 1_1	Wash Buffer 1	800 µL
3	Microtiter deep well 96 plate	Wash 1_2	Wash Buffer 1	800 µL
4	Microtiter deep well 96 plate	Wash 2	Wash Buffer 2	1000 µL
5	KingFisher Flex 96 KF plate	Elution	Elution Buffer	150 µL
6	KingFisher Flex 96 KF plate	Tip plate		

4. Place a Thermo Scientific KingFisher Flex 96 tip comb for deep well magnets on an empty **Tip** plate KingFisher Flex 96 KF plate).
5. Start the **Blood_gDNA_200uL_Flex** protocol with the KingFisher Flex 96 and load the plates according to the instructions on the KingFisher display. After all the plates have been loaded into the instrument, the protocol will begin.

6. Add the **MagJET Magnetic Bead** suspension **resuspended well by vortexing** and **Binding Buffer** (supplemented with ethanol, see page 5) to the **Sample** plate, when the KingFisher Flex pauses at the dispense step after the lysis step (approximately 5 minutes after starting the protocol run).

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	Microtiter deep well 96 plate	Sample	Magnetic beads	25 µL
			Binding Buffer	400 µL

7. Place the **Sample** plate back into the instrument and press **Start**. After the pause, the protocol will continue to the end.
8. When the protocol is completed, remove the plates according to the instructions on the KingFisher Flex display and turn off the instrument. Use the purified DNA immediately in downstream applications or store at -20°C.

Protocol B. Instructions for genomic DNA purification from 1 mL of whole blood using KingFisher Flex and 24 deep well plates.

Note:

- When using the MagJET Whole Blood Genomic DNA Purification Kit for the first time, prepare working solutions of Wash Buffer 1, Wash Buffer 2 and Binding Buffer as described on page 5.
- Transfer the **Blood_gDNA_1mL_Flex** protocol file to the KingFisher instrument before first use. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the BindIt Software for KingFisher Instruments version 3.1 User Manual. The protocol files for MagJET Whole Blood Genomic DNA Kit can be found on product web page on www.thermoscientific.com/onebio

1. Obtain five empty Thermo Scientific KingFisher Flex 24 deep well plates and Tip plate.
2. Prepare the **Sample** plate (KingFisher Flex 24 deep well plate).

Add the following reagents to the Sample plate and leave the plate at room temperature while the other plates are being filled

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	KingFisher Flex 24 deep well plate	Sample	Blood sample	1000 µL
			Lysis Buffer	500 µL
			Proteinase K	60 µL

3. Fill the other plates as follows.

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
2	KingFisher Flex 24 deep well plate	Wash 1_1	Wash Buffer 1	3700 µL
3		Wash 1_2	Wash Buffer 1	3700 µL
4		Wash 2	Wash Buffer 2	3700 µL
5		Elution	Elution Buffer	900 µL

4. Place a Thermo Scientific KingFisher Flex 24 tip comb on a **Tip** plate (empty KingFisher Flex 24 deep well plate).
5. Start the **Blood_gDNA_1mL_Flex** protocol with the KingFisher Flex 24 and load the plates according to the instructions on the KingFisher display. After all the plates have been loaded into the instrument, the protocol will begin.
6. Add the **MagJET Magnetic Beads resuspended well by vortexing** and **Binding Buffer** (supplemented with ethanol, see page 5) to the **Sample** plate, when the KingFisher Flex pauses at the dispense step after the lysis step (approximately 10 minutes after starting the protocol run).

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	KingFisher Flex 24 deep well plate	Sample	Magnetic beads	70 µL
			Binding Buffer	1600 µL

7. Place the **Sample** plates back into the instrument and press **Start**. After the pause, the protocol will continue to the end.
8. When the protocol is completed, remove the plates according to the instructions on the KingFisher display and turn off the instrument. Transfer the eluate (which contains the purified DNA) to a new clean tube, and then close immediately. Use the purified DNA immediately in downstream applications or store at -20°C.

Protocol C. Instructions for DNA purification from 2 mL of whole blood using KingFisher Flex and 24 deep well plates

Note:

- When using the MagJET Whole Blood Genomic DNA Purification Kit for the first time, prepare working solutions of Wash Buffer 1, Wash Buffer 2 and Binding Buffer as described on page 5.
- Transfer the **Blood_gDNA_2mL_Flex** protocol file to the KingFisher instrument before first use. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the BindIt Software for KingFisher Instruments version 3.1 User Manual. The protocol files for MagJET Whole Blood Genomic DNA Kit can be found on product web page on www.thermoscientific.com/onebio

1. Obtain six empty Thermo Scientific KingFisher Flex 24 deep well plates and a Tip plate.
2. Prepare the **Sample plate 1** and **2** (KingFisher Flex 24 deep well plates). The 2 mL blood sample is divided into two different plates, Sample plate 1 and 2. Pipette 1 mL sample into the well on **Sample plate 1** and 1 mL sample into the corresponding well on **Sample plate 2**. For example, a 2 mL blood sample is divided, into wells A1 on **Sample plate 1** and A1 on **Sample plate 2**. Add the following reagents to the **Sample plates 1** and **2**, and leave the plates at room temperature while the other plates are being filled.

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	KingFisher Flex 24 deep well plate	Sample 1	Blood sample	1000 µL
			Lysis Buffer	500 µL
			Proteinase K	60 µL
2		Sample 2	Blood sample	1000 µL
			Lysis Buffer	500 µL
			Proteinase K	60 µL

3. Fill the other plates as follows:

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
3	KingFisher Flex 24 deep well plate	Wash 1_1	Wash Buffer 1	4000 µL
4		Wash 1_2	Wash Buffer 1	4000 µL
5		Wash 2	Wash Buffer 2	4000 µL
6		Elution	Elution Buffer	900 µL

4. Place a Thermo Scientific KingFisher Flex 24 tip comb on a **Tip** plate (KingFisher Flex 24 deep well plate).
5. Start the **Blood_gDNA_2mL_Flex** protocol with the KingFisher Flex 24 and load the plates according to the instructions on the KingFisher display. After all the plates have been loaded into the instrument, the protocol will begin.

6. Add the **MagJET Magnetic Beads resuspended well by vortexing** and **Binding Buffer** (supplemented with ethanol, see page 5) to the **Sample plates**, when the KingFisher Flex24 pauses at the dispense step after the lysis step (approximately 5 minutes after starting the protocol run).

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	KingFisher Flex 24 deep well plate	Sample 1	Magnetic beads	70 µL
			Binding Buffer	1600µL
2		Sample 2	Magnetic beads	70 µL
			Binding Buffer	1600 µL

7. Place the **Sample** plates back into the instrument and press **Start**. After the pause, the protocol will continue to the end.
8. After the run is finished, remove the plates and transfer the eluate (which contains the purified DNA) to a clean and new tube, then close immediately.

When the protocol is completed, remove the plates according to the instructions on the KingFisher display and turn off the instrument. Transfer the eluate (which contains the purified DNA) to a new clean tube, and then close immediately. Use the purified DNA immediately in downstream applications or store at -20°C.

Protocol D. Instructions for genomic DNA purification from 200 µL of whole blood using KingFisher Duo with 12-pin magnet head and Microtiter deep well 96 plate

Note:

- When using the MagJET Whole Blood Genomic DNA Purification Kit for the first time, prepare working solutions of Wash Buffer 1, Wash Buffer 2 and Binding Buffer as described on page 5.
- Transfer the **Blood_gDNA_200uL_Duo** protocol file to the KingFisher instrument before first use. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the BindIt Software for KingFisher Instruments version 3.1 User Manual. The protocol files for MagJET Whole Blood Genomic DNA Kit can be found on product web page on www.thermoscientific.com/onebio
- Ensure you are using the KingFisher Duo 12-pin magnet head and heating block.

1. Obtain one empty Thermo Scientific Microtiter 96 deep well plate and one Thermo Scientific KingFisher Duo elution strip.
2. Prepare the **Blood DNA** plate (Microtiter deep well 96 plate).

Add the following reagents to the rows. Note that row B is reserved for the tip and should be left empty. Note that rows C, D and H are left empty.

Plate name and type	Row	Row name	Content	Sample/reagent volume per well
Blood DNA plate Microtiter deep well 96 plate	A	Sample	Blood sample	200 µL
			Lysis Buffer	100 µL
			Proteinase K	20 µL
	B	Tip	12-tip comb	Empty
	C	Empty	Empty	Empty
	D	Empty	Empty	Empty
	E	Wash 1_1	Wash Buffer 1	800 µL
	F	Wash 1_2	Wash Buffer 1	800 µL
	G	Wash 2	Wash Buffer 2	1000 µL
	H	Empty	Empty	Empty

3. Fill the **KingFisher Duo elution strip** as follows. Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user and the Elution Buffer is pipetted into the correct wells.

Elution strip	Content	Reagent volume per well
KingFisher Duo elution strip	Elution Buffer	130 µL

4. Place a Thermo Scientific KingFisher Duo 12-tip comb into row B on the **Blood DNA** plate.
5. Switch on the KingFisher Duo instrument and ensure you are using the KingFisher Duo 12-pin magnet head and heating block. Start the **Blood_gDNA_200uL_Duo** protocol. Insert the **Blood DNA** plate and **elution strip** into the instrument as indicated on the KingFisher Duo display. Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user.

6. Add the **MagJET Magnetic beads resuspended well by vortexing** and **Binding Buffer** (supplemented with ethanol, see page 5) to row A of the Blood DNA plate.

Plate name and type	Row	Row name	Content	Sample/reagent volume per well
Blood DNA plate Microtiter deep well 96 plate	A	Sample	Magnetic beads	25 µL
			Binding Buffer	400 µL

7. Place the **Blood DNA** plate back into the instrument and press Start. After the pause, the protocol will continue to the end.
8. When the protocol is complete, remove the plate and elution strip according to the instructions on the KingFisher Duo display and turn off the instrument. Transfer the eluate (which contains the purified DNA) to a new clean tube, and then close immediately. The purified DNA is ready for use in downstream applications.

Protocol E. Instructions for genomic DNA purification from 1 mL of whole blood using KingFisher Duo with 6-pin magnet head and 24 deep well plates

Note:

- When using the MagJET Whole Blood Genomic DNA Purification Kit for the first time, prepare working solutions of Wash Buffer 1, Wash Buffer 2 and Binding Buffer as described on page 5.
- Transfer the **Blood_gDNA_1mL_Duo** protocol file to the KingFisher instrument before first use. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the BindIt Software for KingFisher Instruments version 3.1 User Manual. The protocol files for MagJET Whole Blood Genomic DNA Kit can be found on product web page on www.thermoscientific.com/onebio
- Ensure that you are using the KingFisher Duo 6-pin magnet head and heating block.

1. Obtain two empty Thermo Scientific KingFisher Flex 24 deep well plates.
2. Prepare the **Blood DNA plates 1 and 2**. Add the following reagents to the **Blood DNA plates 1 and 2** (KingFisher Flex 24 deep well plates). Pipette 1 mL sample into the wells of row A of the **Blood DNA plate 1**.

Add the following reagents to the rows. Note that row B of the **Blood DNA plate 1** is reserved for the tip and should be left empty. Note that rows C of the **Blood DNA plate 1** and D of the **Blood DNA plate 2** are left empty too.

Plate name and type	Row	Row name	Content	Sample/reagent volume per well
Blood DNA plate 1 KingFisher Flex 24 deep well plate	A	Sample	Blood sample	1000 µL
			Lysis Buffer	500 µL
			Proteinase K	80 µL
	B	Tip	12-tip comb	Empty
	C	Empty	Empty	Empty
	D	Wash 1_1	Wash Buffer 1	3500 µL
Blood DNA plate 2 KingFisher Flex 24 deep well plate	A	Elution	Elution Buffer	900 µL
	B	Wash 2	Wash Buffer 2	5000 µL
	C	Wash 1_2	Wash Buffer 1	3500 µL
	D	Empty	Empty	Empty

3. Place a Thermo Scientific KingFisher Duo 6-tip comb into row B of the **Blood DNA plate 1**.
4. Switch on the KingFisher Duo and make sure that you are using the KingFisher Duo 6-pin magnet head and heating block. Start the **Blood_gDNA_1mL_Duo** protocol. Insert the **Blood DNA plates** into the instrument as indicated on the KingFisher Duo display. After both plates have been loaded into the instrument, the protocol will begin.
5. Add the **MagJET Magnetic beads resuspended well by vortexing** and **Binding Buffer** (supplemented with ethanol, see page 5) to row A of the **Blood DNA plate 1**, when the KingFisher Duo pauses at the dispense step after the lysis step (approximately 10 minutes after starting the protocol run).

Plate name and type	Row	Row name	Content	Sample/reagent volume per well
Blood DNA plate 1 KingFisher Flex 24 deep well plate	A	Sample	Magnetic beads	100 µL
			Binding Buffer	1840 µL

6. Place the **Blood DNA plate 1** back into the instrument and press Start. After the pause, the protocol will continue to the end.
7. When the protocol is complete, remove the plate and elution strip according to the instructions on the KingFisher Duo display and turn off the instrument. Transfer the eluate (which contains the purified DNA) to a new clean tube, and then close immediately. The purified DNA is ready for use in downstream applications.

Protocol F. Instructions for genomic DNA purification from 2 mL of whole blood using KingFisher Duo with 6-pin magnet head and 24 deep well plates

Note:

- When using the MagJET Whole Blood Genomic DNA Purification Kit for the first time, prepare working solutions of Wash Buffer 1, Wash Buffer 2 and Binding Buffer as described on page 5.
- Transfer the **Blood_gDNA_2mL_Duo** protocol file to the KingFisher instrument before first use. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the BindIt Software for KingFisher Instruments version 3.1 User Manual. The protocol files for MagJET Whole Blood Genomic DNA Kit can be found on product web page on www.thermoscientific.com/onebio
- Ensure that you are using the KingFisher Duo 6-pin magnet head and heating block

1. Obtain two empty Thermo Scientific KingFisher Flex 24 deep well plates.
2. Prepare the **Blood DNA plates 1 and 2** by adding the following reagents to the **Blood DNA plates 1 and 2** (KingFisher Flex 24 deep well plates). The 2 mL blood sample is divided into two different wells of rows A and B of the **Blood DNA plate 1**. Pipette 1 mL sample into the well of row A and 1 mL sample into corresponding well of row B. A 2 mL blood sample is divided, for example, into wells A1 and B1 of the **Blood DNA plate 1**.
3. Add the following reagents to the rows. Note that row C of the **Blood DNA plate 1** is reserved for the tip and should be left empty.

Plate name and type	Row	Row name	Content	Sample/reagent volume per well
Blood DNA plate 1 KingFisher Flex 24 deep well plate	A	Sample 1	Blood sample	1000 µL
			Lysis Buffer	500 µL
			Proteinase K	60 µL
	B	Sample 2	Blood sample	1000 µL
			Lysis Buffer	500 µL
			Proteinase K	60 µL
	C	Tip	12-tip comb	Empty
	D	Wash 1_1	Wash Buffer 1	4800 µL
Blood DNA plate 2 KingFisher Flex 24 deep well plate	A	Elution	Elution Buffer	900 µL
	B	Wash 2_2	Wash Buffer 2	5000 µL
	C	Wash 2_1	Wash Buffer 2	5000 µL
	D	Wash 1_2	Wash Buffer 1	4800 µL

4. Place a Thermo Scientific KingFisher Duo 6-tip comb into row C of the **Blood DNA plate 1.**
5. Switch on the KingFisher Duo and make sure that you are using the KingFisher Duo 6-pin magnet head and heating block. Start the **Blood_gDNA_2mL_Duo** protocol. Insert the **Blood DNA plates** into the instrument as indicated on the KingFisher Duo display. After both plates have been loaded into the instrument, the protocol will begin.

6. Add the **MagJET Magnetic beads resuspended well by vortexing** and **Binding Buffer** (supplemented with ethanol, see page 5) to **rows A and B** of the **Blood DNA plate 1**, when the KingFisher Flex pauses at the dispense step after the lysis step (approximately 10 minutes after starting the protocol run).

Plate name and type	Row	Row name	Content	Sample/reagent volume per well
Blood DNA plate 1 KingFisher Flex 24 deep well plate	A	Sample 1	Magnetic beads	70 µL
			Binding Buffer	1600 µL
	B	Sample 1	Magnetic beads	70 µL
			Binding Buffer	1600 µL

7. Place the **Blood DNA plate 1** back into the instrument and press OK. After the pause, the protocol will continue to the end.
8. When the protocol is complete, remove the plates according to the instructions on the KingFisher Duo display and turn off the instrument. Transfer the eluate (which contains the purified DNA) to a new clean tube, and then close immediately. The purified DNA is ready for use in downstream applications.

Protocol G. Instructions for manual genomic DNA purification from 200 µL of whole blood

The following protocol is based on transfer of liquids by pipetting through different purification steps rather than magnetic bead transfer as in KingFisher automatic protocols. This allows the kit to be used in various throughput applications using magnetic rack and manual or automated pipetting equipment. Protocols for the other automated pipetting platforms should be optimized for each platform and sample used. To enable protocol optimization all buffers are available to purchase separately.

Note: When using the MagJET Whole Blood Genomic DNA Purification Kit for the first time, prepare working solutions of Wash Buffer 1, Wash Buffer 2 and Binding Buffer as described on page 5.

1. Add 20 µL **Proteinase K** and 100 µL **Lysis Buffer** to a 200 µL blood sample. Vortex 30 s (at maximum speed) and incubate 3 minutes at room temperature (15-25°C). Vortex 15 s to mix and briefly spin to remove drops from inside of the lid.
2. Add 25 µL **MagJET Magnetic beads resuspended well by vortexing** and 400 µL **Binding Buffer** (supplemented with ethanol, see page 5). Resuspend the magnetic beads by vortexing, briefly spin to remove droplets from inside of the lid. Place the tube on the magnetic rack and let the magnetic beads collect at the magnet for 3 minutes. Discard the supernatant by using a pipette.
3. Remove the magnetic rack and add 800 µL **Wash Buffer 1** (supplemented with ethanol, see page 5). Resuspend the magnetic beads by vortexing, briefly spin to remove drops from inside of the lid. Place the tube on the magnetic rack and let the magnetic beads collect at the magnet for 2-3 minutes. Discard the supernatant by using a pipette.
4. Repeat step 3 using 800 µL of **Wash Buffer 1**.
5. Remove the magnetic rack and add 1000 µL **Wash Buffer 2** (supplemented with ethanol, see page 5). Resuspend the magnetic beads by vortexing, briefly spin to remove drops from inside of the lid. Place the tube on the magnetic rack and let the magnetic beads collect at the magnet for 2-3 minutes. Discard the supernatant by using a pipette. Carefully eliminate supernatant droplets using a small pipette tip and make sure no droplets are left on the tube wall.

Note: Make sure that all the supernatant is completely removed in the last washing step. If there are any visible droplets incubate at room temperature (15-25°C) for 1 minute.

6. Remove the magnetic rack and add 150 µL **Elution Buffer**. Resuspend the magnetic beads by vortexing, incubate for 5 minutes at 72°C, place the tube on the magnetic rack and let the magnetic beads collect at the magnet for 2-3 minutes.
7. While on the magnetic rack, transfer the eluate (which contains the purified DNA) to a new clean tube, then close immediately. Use the purified DNA immediately in downstream applications or store at -20°C.

Protocol H. Instructions for DNA purification from dried blood

1	Cut out the section of filter containing the dried blood sample and place into a microcentrifuge tube.
2	Add 200 µL of 0.9% (w/v) NaCl and incubate 5-10 minutes at room temperature (15-25°C).
3	For manual purification, proceed to **Step 1** of the **Protocol G "Instructions for manual genomic DNA purification from 200 µL of whole blood"** on page 19. For automated purification using KingFisher Flex 96 or KingFisher Duo Instruments proceed to **Step 1** of corresponding **Protocol A "Instructions for genomic DNA purification from 200 µL of whole blood using KingFisher Flex 96 and Microtiter deep well 96 plates"** on page 7 or **Protocol D "Instructions for genomic DNA purification from 200 µL of whole blood using KingFisher Duo with 12-pin magnet head and 96 deep well plate"** on page 13.

Protocol I. Instructions for DNA purification from buffy coat

1	Centrifuge 1.5 mL of whole blood at 2,500 × g (~5,000 rpm) for 10 minutes at room temperature. Three layers should be visible.
2	Remove upper clear layer by aspiration.
3	Collect approximately 200 µL of intermediate layer using an automatic pipette.
4	For manual purification, proceed to **Step 1** of the **Protocol G "Instructions for manual genomic DNA purification from 200 µL of whole blood"** on page 19. For automated purification using KingFisher Flex 96 or KingFisher Duo Instruments proceed to **Step 1** of corresponding **Protocol A "Instructions for genomic DNA purification from 200 µL of whole blood using KingFisher Flex 96 and Microtiter deep well 96 plates"** on page 7 or **Protocol D "Instructions for genomic DNA purification from 200 µL of whole blood using KingFisher Duo with 12-pin magnet head and 96 deep well plate"** on page 13.

Protocol J. Instructions for DNA purification from bone marrow

1	Harvest 20 µL of fresh or frozen bone marrow.
2	For manual purification, proceed to **Step 1** of the **Protocol G "Instructions for manual genomic DNA purification from 200 µL of whole blood"** on page 19. For automated purification using KingFisher Flex 96 or KingFisher Duo Instruments proceed to **Step 1** of corresponding **Protocol A "Instructions for genomic DNA purification from 200 µL of whole blood using KingFisher Flex 96 and Microtiter deep well 96 plates" on** page 7 or **Protocol D "Instructions for genomic DNA purification from 200 µL of whole blood using KingFisher Duo with 12-pin magnet head and 96 deep well plate"** on page 13.

Protocol K. Instructions for DNA purification from urine

1	Add 0.5 mL of 0.5 M EDTA to 4.5 mL of urine (final concentration 50 mM).
2	Centrifuge 10 minutes at 800 × g (~3,000 rpm).
3	Discard the supernatant.
4	Resuspend the pellet in 200 µL of 0.9% (w/v) NaCl.
5	For manual purification, proceed to **Step 1** of the **Protocol G "Instructions for manual genomic DNA purification from 200 µL of whole blood"** on page 19. For automated purification using KingFisher Flex 96 or KingFisher Duo Instruments proceed to **Step 1** of corresponding **Protocol A "Instructions for genomic DNA purification from 200 µL of whole blood using KingFisher Flex 96 and Microtiter deep well 96 plates"** on page 7 or **Protocol D "Instructions for genomic DNA purification from 200 µL of whole blood using KingFisher Duo with 12-pin magnet head and 96 deep well plate"** page 13.

Protocol L. Instructions for DNA purification from swabs

1	To collect a sample, scrape the swab 5-6 times against the inside cheek.
2	Swirl the swab for 30-60 s in 200 µL of 0.9% (w/v) NaCl.
3	For manual purification, proceed to **Step 1** of the **Protocol G "Instructions for manual genomic DNA purification from 200 µL of whole blood"** on page 19. For automated purification using KingFisher Flex 96 or KingFisher Duo Instruments proceed to **Step 1** of corresponding **Protocol A "Instructions for genomic DNA purification from 200 µL of whole blood using KingFisher Flex 96 and Microtiter deep well 96 plates"** on page 7 or **Protocol D "Instructions for genomic DNA purification from 200 µL of whole blood using KingFisher Duo with 12-pin magnet head and 96 deep well plate"** page 13.

Lysis Buffer for MagJET Blood gDNA Kit

Xn Harmful

Hazard-determining components of labelling: guanidinium chloride

Risk phrases:

22 Harmful if swallowed.

36/38 Irritating to eyes and skin.

Safety phrases:

23 Do not breathe gas/fumes/vapour/spray.

26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

36/37 Wear suitable protective clothing and gloves.

60 This material and its container must be disposed of as hazardous waste.

Binding Buffer for MagJET Blood gDNA Kit

Xn Harmful

O Oxidizing

Hazard-determining components of labelling: sodium perchlorate

Risk phrases:

22 Harmful if swallowed.

9 Explosive when mixed with combustible material.

Safety phrases:

17 Keep away from combustible material.

23 Do not breathe gas/fumes/vapour/spray.

27 Take off immediately all contaminated clothing.

36 Wear suitable protective clothing.

60 This material and its container must be disposed of as hazardous waste.

Wash Buffer 1 (conc.) for MagJET Blood gDNA Kit

O Oxidizing

Hazard-determining components of labelling: sodium perchlorate

Risk phrases:

9 Explosive when mixed with combustible material.

Safety phrases:

17 Keep away from combustible material.

27 Take off immediately all contaminated clothing.

60 This material and its container must be disposed of as hazardous waste.

Proteinase K

Xn Harmful

Hazard-determining components of labelling: Proteinase, Tritirachium album serine

Risk phrases:

42 May cause sensitisation by inhalation.

Safety phrases:

23 Do not breathe gas/fumes/vapour/spray.
36 Wear suitable protective clothing.
45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
60 This material and its container must be disposed of as hazardous waste.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

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